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Cassava Peels – A Potential Co-substrate for the Biodegradation of Low-Density Polyethylene (LDPE) using Locally Isolated Bacteria and Fungi

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Abstract— *The objective of this research was to study the potential of using cassava peels, an agro-industrial waste as co-substrate to enhance the biodegradation of Low-Density Polyethylene (LDPE). The microorganisms used for biodegradation were eight bacterial isolates including Pseudomonas aeruginosa(MAK1), Pseudomonas aeruginosa(ABJ6), Bacillus megaterium, Providencia stuarti, Alcaligenes feacalis, Enterobacter hormaechei, Klebsiella pnuemonia and Proteus vulgaris, and eight fungal isolates including Aspergillus flavus, Aspergillus niger, Fusarium chlamydosporium, Trichoderma viride, Mucour indicus, Rhizopus miehei, Basidobolus ranarum and Microsporum nanum, which were previously isolated and screened for their ability to utilize LDPE and stored at 4°C in the Microbiology laboratory of Nasarawa State University, Keffi, Nasarawa State, Nigeria. Biodegradation was evaluated in a sterilized Mineral Salts Medium (MSM) containing 0.500g waste LPDE strips (1cm by 5cm) in a 500 milliliter flask. 600µl aliquot of each microbe and Cassava Peel Powder (CPP) at a final concentration of 0.1%(w/v) were added into separate flasks and incubated at 30°C in a rotary shaker for eight (8) weeks. Control experiment was set up without CPP and another without both the microbe and CPP. Biodegradation was assessed gravimetrically by measuring the weight loss of the LDPE strips two weekly during the incubation period. The results showed that there was a significant increase (at 95% confidence level) in the percentage weight loss of the LDPE strips with CPP as co-substrate and the highest weight loss was recorded for Pseudomonas aeruginosa1 at 95.2±0.34% as against 19.84±0.04% without CPP as co-substrate. Likewise, there was a significant increase (at 95% confidence level) in LDPE strip weight loss by all the fungal isolates with the highest weight loss recorded for Aspergillus flavus at 80.2±0.54% as against 19.40±0.14% in the absence of CPP. This work reveals that cassava peels could be utilized as potential enhancer for the biodegradation of LDPE in the environment.*

Keywords— *Low-density polyethylene, Cassava peel, Biodegradation, Co-substrate, fungi, bacteria, weight loss.*

I. INTRODUCTION

Low-Density Polyethylene (LDPE) is a light versatile synthetic resin made from the polymerization of ethylene. It is a member of the important family of polyolefin resins and the most widely used plastic in the world, with very high level of hydrophobicity, high molecular weight and very low biodegradability (Muhonja *et al*., (2018); Tiago *et al*., (2023)).

LDPE packaging is used on a daily basis around the globe because of its easy processing for various products such as bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs and water pipes and because of its durability (Begum *et al.,* 2015). Low –Density Polyethylene (LDPE) accounts for 60% of the total plastic production and the most commonly found solid waste (Raaman *et al*., 2012; Das & Kumar, 2014; Gajendiran *et al*., 2016). As carrier and grocery bags, LDPE poses a great disposal challenge because it can take up to 1000 years to degrade naturally (Sangale *et al.,* 2012; Muhonja *et al*., 2018), only a fraction of this is recycled whereas most of the wastes enter into the landfills (Grover e*t al*., 2015). The global amount of polyethylene plastic is increasing by 12% per year, with 0.15 billion tons of synthetic polymers developed each year (Abdullah *et al*., 2022), this raises huge environmental concern. Polyethylene which is mostly the packaging plastic constitutes 10% of the total municipal waste generated around the globe (D'Alessandro, 2014; Mallisetty *et al*., 2023). The current state of plastic (Polyethylene) bag waste pollution in Nigeria is alarming with several environmental impacts requiring urgent attention (Ogwo *et al.,* 2013; Abioye *et al*., 2018).

The use of and waste treatment of plastics have become a global problem. Therefore, it is inevitable necessity to minimize polyethylene and other plastics and to develop efficient disposal methods or combination of both (Restrepo-Flore *et al*., 2014; Ghatge *et al*., 2020).

There are limited methodologies available for reutilization of plastic wastes. Such examples are waste minimization and recycling, landfill, incineration, gasification and hydrogenation. However, the dumping of waste plastic in open areas is still the most commonly used disposal methods for municipal solid waste in developing countries like Nigeria (Awashti *et al*., 2017; Abioye *et al*., 2018).

Although, various polyethylene degradation methods such as photo-degradation, thermo-oxidation are available, but the cheapest, eco-friendly and adequate method is biodegradation (Białowiec, 2011; Ghatge *et al*., 2020; Yao *et al*., 2022). Biodegradation of polyethylene (plastics) is a natural process of degrading materials through microbes such as bacteria, fungi and algae. It involves only microbial agents and not heat (Priyanka & Archana, 2011; Sangale *et al*., 2012; Munhoja *et al*., 2018).

The use of agro-industrial wastes such as cassava peels, pineapple peels and coconut husk ash in the bioremediation of soil pollutants such as hydrocarbon petroleum products have been variously studied (Dhanasekaran *et al*., 2011; Patel, 2012; Al-Wasify *et al*., 2014 and Kormin et al., 2017).

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Cassava peels has high starch content that is biodegradable, inexpensive and abundantly available with high starch content (Otache *et al*., 2021). They are produced in large quantities in Nigeria and are indiscriminately dumped in the environment. For example, about 2.96 million metric tons of cassava peels are generated and discarded annually in Nigeria from about 10 million metric tonnes of cassava processed for garri (a staple Nigerian food) alone (Aro *et al*., 2010; Mustapha *et al*., 2019; Onuoha *et al*., 2020). They serve as co-pollutants with polyethylene plastic wastes and constitute huge environmental nuisance especially as their organic nature makes them highly degradable by microorganisms usually releasing foul odor in the environment where they are dumped and also end up polluting the surface and underground water (Dhanasekaran *et al*., 2011; Odunfa & Olanbiwoninu 2012; Onuoha *et al*., 2020).

Nigeria needs to explore the abundant agricultural wastes (Mustafa *et al*., 2019) such as cassava peels, to convert them to useful resource and for environmental sustainability. The aim of this study was to explore the use of cassava peels, an agro-industrial waste as co-substrate to enhance the biodegradation of low-density polyethylene in the environment.

II. Materials And Methods

2.1 Mineral Salt Media (MSM) content:

In one liter of deionized water: K_2HPO_4, 0.5g, KH_2PO_4, 0.04g, NaCl, 0.1g, $CaCl_2.2H_2O$, 0.002g, $(NH_4)_2SO_4$, 0.2g, $MgSO_4.7H_2O$, 0.02g, $FeSO_4$, 0.001g, Agar, (optional), 20.0 g, pH 7.0 ± 0.2.

2.1.1 Nutrient Basal Media content:

The basal salts mineral media used contained the following elements (prepared in distilled water): 12.5g/l K_2HPO_4; 3.8/l KH_2PO_4; 1.0g/l $(NH_4)2SO_4$; 0.1g/l $MgSO_4.7H_2O$ and 5ml trace element solution contain each of the following elements (prepared in distilled water): 0.232g/lH_3BO_3;0.174g/l,$ZnSO_4.7H_2O$;0.116g/l$FeSO_4(NH_4)2SO_4.6H_2O$;0.096g/l$CoSO_4.7H_2O$;0.022g/l$(NH_4)6Mo7O_2.4H_2O$;8.0mg/lCuSO4.5H2O; 8.0mg/l$MnSO_4.4H_2O$. Each medium was prepared using standard microbiological procedures.

2.2 Sample collection and preparation: Cassava waste peels were obtained by peeling the back of the cassava bought from fruit sellers and prepared using the method of Olutosin *et al*. (2021). The peels were washed thoroughly under running tap to remove sands, dirt and other unwanted materials. The cassava peels were then dried in the oven at 55°C for 14 days until crisp. The dried peels were then pulverized into powder using the laboratory blender. The powder was sieved to remove fibres and particles.

2.3 Source of Microorganisms used: Bacteria (*Pseudomonas aeruginosa*(MAK1), *Pseudomonas aeruginosa*(ABJ6), *Bacillus megaterium*, *Providencia stuarti*, *Alcaligenes feacalis*, *Enterobacter hormaechei*, *Klebsiella pnuemonia* and *Proteus vulgaris*, and fungi *(Aspergillus flavus*, *Aspergillus niger*, *Fusarium chlamydosporium*, *Trichoderma viride*, *Mucour indicus*, *Rhizopus miehei*, *Basidobolus ranarum* and *Microsporum nanum)* were collected from slants stores at 4°C in the Microbiology Laboratory of Nasarawa state university. They were previously isolated and characterized from plastic contaminated soils from different parts of North Central Nigeria (Ogu *et al*., 2023).

2.4 Waste LDPE bag preparation and Culture condition.

A method described by Kyaw & Champakalakshmi, (2012), was used in preparing waste polyethylene. Polyethylene films were collected from dump sites inside Nasarawa State University campus, Keffi. These were cut into (5cm X 1cm) strips and then washed first with tap water to remove all debris and soil particles. Then, they were washed with 70% ethanol for 30 minutes, washed with distilled water and subsequently dried in incubator at 60°C before exposure to the microbial isolates. Inoculation and incubation was carried out under aseptic conditions.

2.5 Determination of Biodegradation Levels by Microbial Isolates

2.5.1 Inoculation of the Microbial Isolates for Biodegradation Tests

Using falcon tubes, 30ml of basal mineral medium and 600µl of the microbial stock about 24 days old was mixed with 0.500g of the LDPE strips (Kathiresan, 2003; Kyaw and ChampakLakshmi, (2012). The tubes were incubated at 37°C, and lid was slightly opened for aeration. The tests were performed in triplicate for each isolate. The strips were removed at 2, 4, 6, and 8 weeks after incubation and checked gravimetrically for weight changes. There were a set of control experiments in the test tubes containing only the LDPE strips in the medium devoid of any microbial inoculum.

2.5.2 Weight Loss Measurements

The polyethylene films after exposure to each of the microbial isolates were taken and washed thoroughly with a 2% (v/v) aqueous Sodium Dodecyl Sulphate (SDS) solution for 4 hours. The strips were dried at 60°C overnight in an incubator and placed on a filter paper before weighing with a microbalance, and the percentage weight loss was determined using the following formula: Weight loss (%) = initial weight – final weight/initial weight x 100 (Hadad *et al*., 2005; Kyaw and ChampakLakshmi, 2012).

2.5.3 Biodegradation of LDPE Mixed with Cassava Peel Powder (CPP) (Weight loss measurement)

Using falcon tubes, 30ml of the basal mineral medium and 600µl of the bacterial or fungal stock about 24 days old was mixed with 0.500g of the low-density polyethylene films (Tadros *et al*., 1999; Kathiresan, 2003) and the cassava peel powder at a final concentration of 0.1 % (w/v). The initial concentration of the microbial isolates was about 0.5 McFarland Standard (1.5 x 10^8 colony forming units (CFU/mL).

The tubes were incubated on a rotary shaker (120rpm) at 37°C, and the lid slightly opened for aeration (Kyaw & Champak Lakshmi, 2012). Weight loss of the LDPE strips were measured in triplicates for each isolate. The LDPE films were removed at 2, 4, 6 and 8 weeks after incubation and checked for weight changes.

The polyethylene films after exposure to each LDPE degrading microbial isolate (Bacteria, and fungi), was taken and washed thoroughly with a 2% (v/v) aqueous Sodium Dodecyl

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Sulphate (SDS) solution for 4 hours. The strips were then dried at 60ºC overnight in an incubator and placed on a filter paper before weighing with a microbalance (Mettler Toledo - Balance XPR2U). The percentage weight loss was determined using the following formula:

Weight loss (%) = initial weight – final weight/initial weight x 100 (Hadad *et al*., 2005; Kyaw & ChampakLakshmi, 2012) Results for all the microbial isolates were recorded and analyzed.

2.6 Statistical Analysis

All analysis was conducted in triplicates and analyzed using Microsoft Excel Windows 10 program and Smith Statistical Package (SSP) version 3.1 was used to conduct one-way ANOVA (Gong *et al*., 2023), with significance determined at 95% confidence level. Results was presented as means ± standard error of the mean.

III. Results

3.1 Biodegradation of untreated LDPE waste strips by bacterial isolates

Biodegradation of untreated LDPE strips by bacteria and fungi isolated from soil from dump sites as evidenced by weight loss of the strips are shown in Tables 3.1 and 3.2 (Figures 3.1 and 3.2). The percentage weight reduction of LDPE waste by bacterial isolates between 2 and 8 weeks of exposure were within the range of 0.0-19.80±0.04% and the highest percentage reduction was at 8 weeks duration for *P. aeruginosa* (MAK1: 19.80±0.04%), *P. aeruginosa* (ABJ6: 19.40±0.08%) and *Providencia staurti* (19.20±0.42%) at 2-6 weeks durations for *Klebsiella pneumoniae*, the percentage weight reductions ranged between 0.60±0.17 and 1.40±0.02% between 2 and 8 weeks duration; for *Proteus vulgaris*, the percentage weight reductions ranged from 0.0 to 0.80±0.00% as shown in figures 3.1. There was a significant increase (at 95% confidence level) in LDPE weight loss as time of incubation increased for *P.aeruginosa* (MAK1 and ABJ6), *B.megaterium*, *Providencia stuarti*, and *Proteus vulgaris* as shown in Table 3.1.

The differences in the weight loss of low density polythene were statistically significant between *Pseudomonas. aeruginosa* (MAK1)) or *Pseudomonas. aeruginosa* and *Proteus. vulgaris*

TABLE 3.1 Measurement of biodegradation of LDPE by bacterial isolates over 8 -week incubation period

Bacteria	Initial weight of LDPE strip(g)	Percentage weight loss of LDPE films over time (%) (weeks)			
		2	4	6	8
Control	0.500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Pseudomonas aeruginosa(MAK1)	0.500	9.60±0.18^{ba}	12.80±0.41 ^{ba}	14.20±0.09^{ba}	19.80±0.04^{ba}
Pseudomonas aeruginosa(ABJ6)	0.500	11.00±0.10^{ba}	13.20±0.30 ^{ba}	18.80±0.01^{ba}	19.40±0.08^{ba}
Bacillus megaterium	0.500	5.40±0.20^{b}	11.60±0.61 ^{ba}	13.20±0.04^{ba}	13.40±0.10^{ba}
Providencia stuarti	0.500	6.60±0.11^{b}	8.20±0.41^{b}	17.40±0.001^{ba}	19.20±0.42^{ba}
Alcaligenes faecalis	0.500	6.20±0.12^{b}	6.80±0.12 ^{b}	7.60±0.21^{b}	8.00±0.81^{b}
Enterobacter hormaechei	0.500	3.60±0.21^{b}	5.40±0.01^{b}	5.60±0.11^{b}	5.80±0.31^{b}
Klebsiella pneumonia	0.500	0.60±0.17^{ba}	1.20±0.17 ^{ba}	1.40±0.19 ^{b}	1.40±0.02^{b}
Proteus vulgaris	0.500	0.00±0.00^{ba}	0.60±0.00 ^{ba}	0.60±0.00 ^{ba}	0.80±0.00^{ba}

ba=significance; b=insignificance

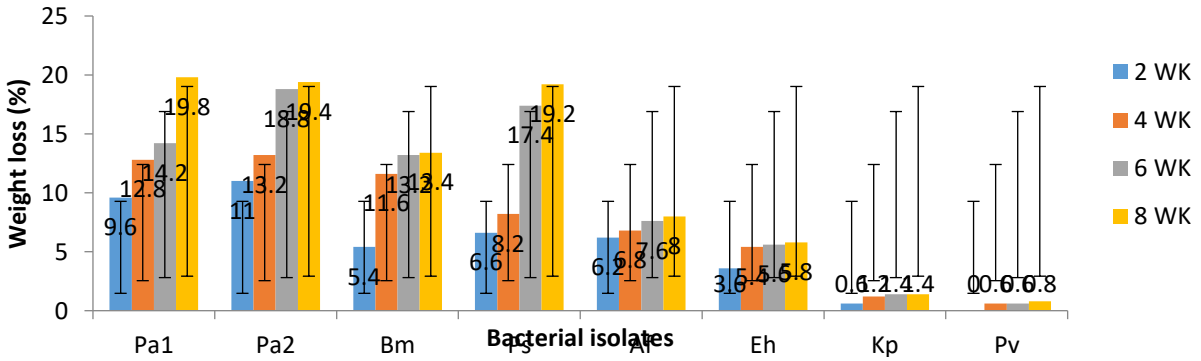

Figure 3.1: Percentage weight loss of waste LDPE films by Bacteria isolated from dump sites in parts of North Central Nigeria

Key; Pa1 –*Pseudomonas aeruginosa*(MAK1), Pa2-*P.aeruginosa* (ABJ6), Bm- *Bacillus megaterium*, Ps-*Providencia stuarti*, Af- *Alcaligenes faecalis*, Eh- *Enterobacter hormaechei*, Kp – *Klebsiella pnuemoniaee*, Pv- *Proteus vulgari*

3.2 Biodegradation of untreated LDPE waste strips by Fungal isolates

The percentage weight reduction of the LDPE strips increased over time for all the fungal isolates, however, this was not significant at 95% confidence level (Table 3.2). The highest weight reduction was recorded after 8 weeks' incubation for *Aspergillus niger* and *Aspergillus flavus* (19.40±0.18% and 19.40±o.14%) respectively, but lowest after 8 week incubation for *Mucour indicus* (8.60±0.11%) and *Rhizopus miehei* (5.80±0.31%) respectively as shown in Figure 3.2. There was no weight reduction recorded for the LDPE waste strips in the control experiment.

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TABLE 3.2: Measurement of biodegradation of LDPE by fungal isolates over 8-week incubation period

Fungi	Initial weight of LDPE strip(g)	Percentage weight loss of LDPE films over time (weeks) (g/g)			
		2	4	6	8
Control	150.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Aspergillus flavus	150.0	3.40±0.08^{b}	5.40±0.41^{b}	14.20±0.19^{b}	19.40±0.14^{b}
Aspergillus niger	150.0	4.20±0.12^{b}	7.20±0.10^{b}	14.20±0.01^{b}	19.40±0.18^{b}
Fusarium chlamydosporium	150.0	6.00±0.10^{b}	7.00±0.21^{b}	11.20±0.28^{b}	12.60±0.10^{b}
Trichoderma sp.	150.0	4.60±0.17^{b}	6.80±0.01^{b}	10.80±0.01^{b}	10.60±0.02^{b}
Mucor indcus	150.0	5.40±0.19^{b}	6.20±0.12^{b}	6.60±0.18^{b}	8.60±0.11^{b}
Rhizopus miehei	150.0	3.60±0.21^{b}	5.40±0.01^{b}	5.60±0.11^{b}	5.80±0.31^{b}
Basidobolus ranarum	150.0	3.40±0.07^{b}	4.40±0.17^{b}	5.00±0.19^{b}	5.20±0.05^{b}
Microsporum nanum	150.0	4.00±0.03^{b}	5.20±0.09^{b}	5.20±0.10^{b}	6.00±0.0^{b}

b=insignificant

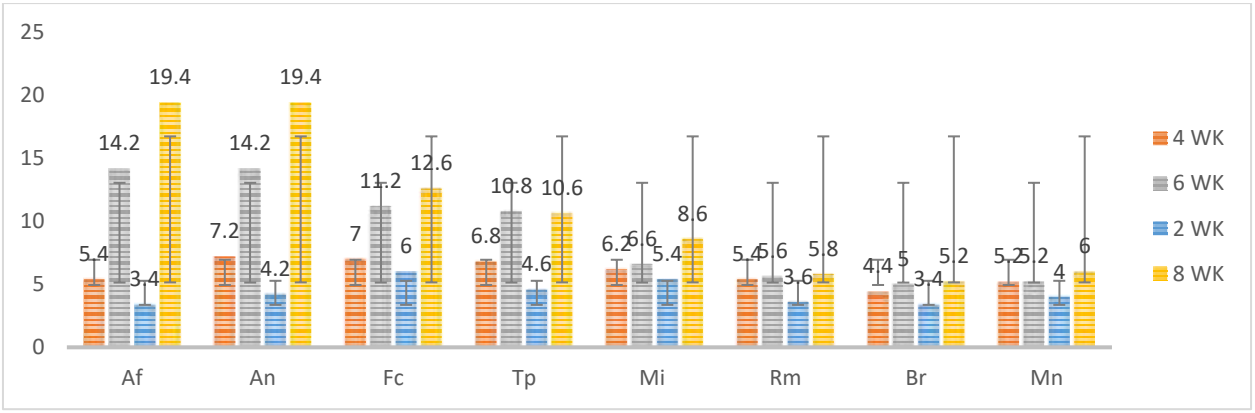

Figure 3.2: Percentage weight loss of waste LDPE films by Fungi isolated from dump sites in relation to duration of incubation in weeks

Fungal Isolates

Key; Af- *Aspergillus flavus*, An – *Aspergillus niger*, Fc – *Fusarium chlamydosporium*, Tp – *Trichoderma sp*, Mi – *Mucor indicus*, Rm – *Rhizopus miehei*, Br – *Basidiopolus ranarum*, Mn – *Microsporium nanum*

3.4 Effect of Cassava Peel Powder (CPP) on Biodegradation of Low-Density Polyethylene (LDPE) Wastes Strips

3.4.1 Effect of Cassava Peel Powder (CPP) on Biodegradation of Low-Density Polyethylene (LDPE) Wastes Strips by Bacterial Isolates

The effect of incorporating an agricultural waste, Cassava (*Manihot esculenta Crantz*) on degradation of LDPE waste strips by bacteria and fungi isolated from soil from dumpsites in parts of North Central Nigeria are shown in Tables 3.3 and 3.4,(Figures 3.3 and 3.4). The effect of adding cassava peels into the incubating medium shows that the bacterial isolates namely; *Pseudomonas aeruginosa* (MAK1), *Pseudomonas aeruginosa* (ABJ6), *B. megaterium* and *Providencia stuarti* degraded more LDPE waste strips with percentage weight reduction ranges of 42.4 to 95.2%, 40.0to 83.2%, 40.0 to 89.0% and 26.0 to 82.2% at 2 to 8 weeks of incubation, while *Klebsiella pneumoniae* and *Proteus vulgaris* degraded less of LDPE waste strips in comparison with percentage weight reduction ranges of 16.0%-33.0% and 17.6%-33.4% at 2-8 weeks' exposure as shown in Table 3.3 and Figure 3.3 respectively. Generally, addition of cassava peels into the incubating medium significantly increased biodegradation activity of all bacterial isolates at 95% confidence level between 6 to 8weeks (Table 3.3).

TABLE 3.3 Effect of Cassava Peels (enhancer) on LDPE waste degradation by bacterial isolates

Bacteria	Weight loss in weeks (%)				
	Initial weight (g)	2	4	6	8
Control	150.0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Pseudomonas aeruginosa1	150.0	42.4±0.11^{b}	42.4±0.11^{b}	82.0±0.29^{ab}	95.2±0.34^{ab}
Pseudomonas aeruginosa2	150.0	40.0±0.21^{b}	40.0±0.21^{b}	82.8±0.41^{ab}	83.2±0.38^{ab}
Bacillus megaterium	150.0	40.0±0.30^{b}	40.0±0.30^{b}	80.0±0.74^{ab}	89.0±0.50^{ab}
Providencia stuarti	150.0	26.0±0.11^{b}	26.0±0.11^{b}	80.4±0.51^{ab}	82.2±0.42^{ab}
Alcaligenes faecalis	150.0	22.6±0.32^{b}	22.6±0.32^{b}	40.2±0.23^{ab}	44.4±0.81^{ab}
Enterobacter hormaechei	150.0	18.8±0.01^{b}	18.8±0.01^{b}	36.0±0.51^{ab}	41.4±0.61^{ab}
Klebsiella pneumonia	150.0	16.0±0.07^{b}	16.0±0.07^{b}	26.8±0.19^{ab}	33.0±0.52^{ab}
Proteus vulgaris	150.0	17.6±0.08^{b}	17.6±0.08^{b}	25.8±0.23^{ab}	33.4±0.44^{ab}

b=insignificance; ab=significance

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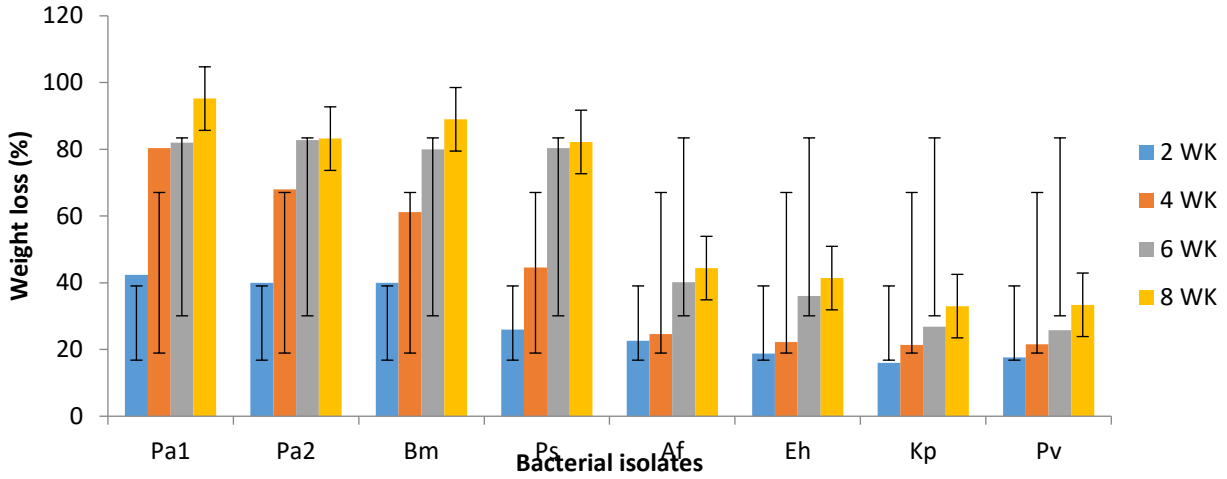

Figure 3.3 Effect of Cassava Peels on Percentage Weight loss of waste LDPE films by bacteria isolated from dump sites

Key; Pa1 –*Pseudomonas aeruginosa* (MAK1), Pa2- *P.aeruginosa* (ABJ6), Bm- *Bacillus megaterium*, Ps- *Providencia stuarti*, Af- *Alcaligenes faecalis*, Eh- *Enterobacter hormaechei*, Kp – *Klebsiella pnuemoniaee*, Pv- *Proteus vulgaris*

3.4.2 Effect of Cassava Peel Powder (CPP) on Biodegradation of Low-Density Polyethylene (LDPE) Wastes Strips by Fungal Isolates

Similarly, the fungal isolates namely, *Aspergillus flavus*, *A. niger* and *Fusarium chlamydosporium* when incubated with LDPE in a medium containing cassava peels degraded more LDPE waste strips at 4-8 weeks' incubation with percentage weight reduction of LDPE ranging from 42.8% to 80.2% as shown in Table 3.4. *Microsporum nanum* had the lowest percentage weight reduction of LDPE waste with percentage weight reduction ranging from 22.6% to 42.0% respectively as shown in Table 3.4 (Figure 3.4). All fungal isolates showed significant biodegradation activity (at 95% confidence level) after 8weeks incubation with the incorporation of cassava peels into the incubating medium (Table 3.4).

TABLE 3.4: Effect of cassava peels (enhancer) on degradation of LDPE wastes by fungal isolates.

Fungi		**Weight loss in weeks (%)**			
	Initial weight	**2**	**4**	**6**	**8**
Control	0.500	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Aspergillus flavus	0.500	50.2±0.20^{ab}	62.0±0.20 ^{ab}	64.2±0.35 ^{ab}	80.2±0.54 ^{ab}
Aspergillus niger	0.500	50.0±0.41 ^{ab}	73.4±0.26 ^{ab}	66.0±0.41 ^{ab}	73.6±0.11 ^{ab}
Fusarium chlamydosporium	0.500	40.2±0.62 ^{ab}	60.0±0.51 ^{ab}	61.4±0.04 ^{ab}	80.0±0.35 ^{ab}
Trichoderma sp.	0.500	50.2±0.31 ^{ab}	42.8±0.21 ^{ab}	50.2±0.54 ^{ab}	62.2±0.21 ^{ab}
Mucor indcus	0.500	28.6±0.02 ^{ab}	34.0±0.41 ^{ab}	40.2±0.84 ^{ab}	48.4±0.61 ^{ab}
Rhizopus miehei	0.500	22.2±0.41 ^{ab}	30.2±0.10 ^{ab}	40.2±0.54 ^{ab}	55.4±0.40 ^{ab}
Basidiobolus ranarum	0.500	20.0±0.32 ^{ab}	24.2±0.06 ^{ab}	40.2±0.30 ^{ab}	44.2±0.30 ^{ab}
Microsporum nanum	0.500	22.6±0.21 ^{ab}	28.0±0.10 ^{ab}	32.0±0.20 ^{ab}	42.0±0.10 ^{ab}

ab=significance

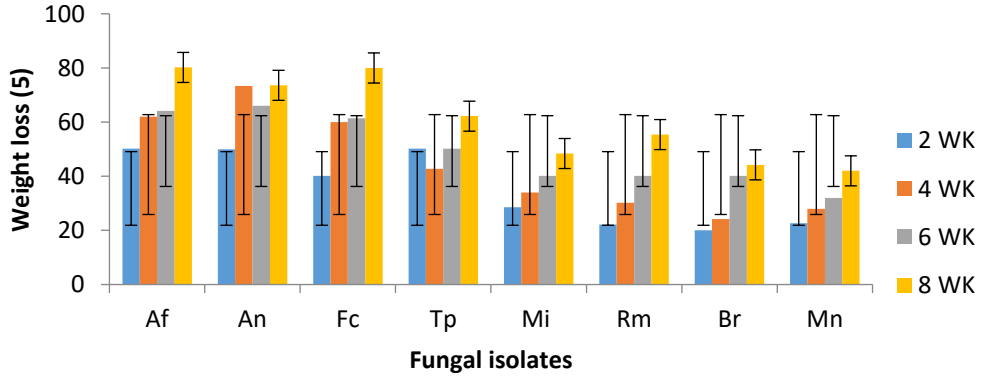

Figure 3.4 Effect of Cassava Peels on Percentage Weight loss of waste LDPE films by fungi isolated from dump sites

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Key; Af- *Aspergillus flavus*, An – *Aspergillus niger*, Fc – *Fusarium chlamydosporium*, Tp – *Trichoderma sp*, Mi – *Mucor indicus*, Rm – *Rhizopus miehei*, Br – *Basidiopolus ranarum*, Mn – *Microsporium nanum*

IV. Discussion

After eight weeks of incubation, weight loss of LDPE strips incubated with bacterial isolates ranged from 0.8% for *Proteus vulgaris* to 19.8% for *Pseudomonas aeruginosa* (MAK1). There was no weight loss observed in control experiment, confirming the action of the bacterial isolates. Among the eight bacterial isolates used for the biodegradation experiments, *Pseudomonas aeruginosa* (MAK1), *P. aeruginosa* (ABJ6), *Providencia stuarti, Proteus vulgaris,* and *Bacillus megaterium* showed significant increase in activity (at 95% confidence level) after 8 weeks' incubation and were the most effective in degrading LDPE strips. This is in line with results obtained from previous studies by Deepika and Mahduri (2015), which reported that *Pseudomonas* species had significant plastic degradation capacity, degrading up to 24.22% of plastic polymer within a period of 6 months. Similarly, Kyaw *et al.* (2012) studied the biodegradation of LDPE by *Pseudomonas* species and reported that after 120 days of incubation, the percentage weight reduction was 20% in *Pseudomonas aeruginosa* (PAO1), 11% in *Pseudomonas aeruginosa* (ATCC) strain, 9% in *Pseudomonas putida* and 11.3% in *Pseudomonas syringae* strain. Badrimarayanan (2015) also reported that *Pseudomonas alcaligenes* exhibited significant polyethylene degradation ability.

Similarly, Ojiego *et al.* (2022) reported that about 6 out of the bacterial isolates from dump sites in Abuja, Nigeria investigated for plastic degradation, only two genera, *Providencia* and *Proteus* were found to be the best degraders of plastic materials under controlled conditions. Wanjohi *et al.* (2018) also reported biodegradation activities of *Providencia* sp. and *Proteus* sp., however the rate of degradation attributed to these bacterial species in this study was higher than that reported by Ojiego *et al*. (2022) and Wanjohi *et al*. (2018). These variations may be attributed to the differences in the bacterial species / strain and in the molecular weights of the plastics used in the biodegradation studies (Ojiego *et al.*, 2022).

According to Ru *et al.* (2020), molecular weight of the polymer / plastic materials generally affects their physical properties such as solubility and surface areas, which in turn determine the rates of biodegradation and valorization by microorganisms. In a different study by Asmita *et al.* (2015), *Bacillus subtilis* and *Pseudomonas aeruginosa* were identified as potential biodegraders of the polyethylene terephthalate (PET) and Polystyrene (PS) which are important plastic materials. These results are similar to the results obtained for *Bacillus* and *Pseudomonas* species isolated in this study.

Biodegradation of plastic materials occurs through the activities of species-specific microbial enzymes (Ru *et al.*, 2020). Recently, Mohanan *et al*. (2020), and Shilpa & Meena (2022) reported that upon microbial exposure to any plastic material, and depending on the molecular weights, chemical structure, and crystalline nature of this plastic, the microbes release special extracellular enzymes, which adsorbs to the polymer surface stepwise followed by hydroperoxidation and then hydrolytic cleavage until mineralization occurs. Only microbes that possess these enzymes and in the presence of optimum environmental conditions and nutrient substrates, can efficiently breakdown the plastic polymers (Ojiego *et al*., 2022). Reduction in weight may also be due to the consumption of LDPE films as sole carbon source by the bacterial isolates, confirming these organisms' capacity in degrading LDPE.

Likewise, the degradation of LDPE films by the various fungal isolates from this study were also time-dependent. After eight weeks of incubation, highest weight loss was recorded from *Aspergillus flavus* and *A. niger* (19.4% respectively, followed by *Fusarium chlamydosporium* (12.60%) whilst the lowest was observed for *Rhizopus* sp (5.80%) and *Basidiobolus ranarum* (5.2%). Fungal species from the genus, *Aspergillus* especially, *A. niger, A. flavus,* and *A. oryzae* are usually employed in the degradation of LDPE due to its ability to freely and abundantly grow in soil and garbage sites and due to its shorter incubation time when compared with other fungal species (Bosshard, 2011).

Mendez *et al.* (2007) also isolated *Aspergillus flavus* from sanitary landfills and found that it degraded polyethylene. The decrease in weight is in association with other findings (Gilan *et al.,* 2004; Manzur *et al*., 2004; Singh *et al.,* 2012), who also carried out degradation of LDPE using *Aspergillus* and *Penicillium* species. A higher weight loss recorded for the fungi species in this study can be attributed to the breakdown of the carbon backbone by fungal enzymes (Dsouza *et al*., 2021).

The resultant monomers and oligomers are used directly by the microorganisms as a carbon source (Kathiresan, 2003). The loss of weight in the polyethylene samples investigated by DSouza *et al*. (2021) was also attributed to the formation of biofilms over the LDPE strips which decreased the hydrophobicity and contact surface between the microbes and LDPE samples.

Similar fungi species as implicated in this study such as *Aspergillus* sp., *Mucor* sp. and *Rhizopus* sp. have been variously reported elsewhere as solid waste biodegraders (Douglas *et al.*, 2020; Ayeni *et al*., 2022). The fungal isolates' degrading ability was also thought to be as a result of their ability to survive better in static- solid medium (Ayeni *et al.,* 2022). Generally, biodegradation gives rise to loss of polymer integrity and weight loss; the loss observed is proportional to the surface area since biodegradation usually is initiated at the surface of the polymer (Gajendiran *et al.,* 2016).

The effect of adding an agro-industrial waste (Cassava Peels Powder) into the mineral salts medium with LDPE strips used for biodegradation showed remarkable results for both bacterial and fungal isolates after 8 weeks' incubation.

All the bacterial isolates showed significant LDPE degradation in terms of percentage weight loss of LDPE strips with cassava peels in the medium. The highest weight reduction of 95±0.34% was recorded for *Pseudomonas aeruginosa* (MAK1), 89.0±0.5% for *Bacillus megaterium* and 83.2±0.38% for *Pseudomonas aeruginosa* (ABJ6) as against 19.80%, 13.40% and 19.2% LDPE weight reduction after 8

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weeks' incubation respectively without cassava peels in the medium.

The fungal isolates showed a similar pattern in LDPE degradation with the incorporation of cassava peels in the medium after 8 weeks' incubation. The high performing fungal isolates recorded LDPE weight reductions of 80.2±0.54% for *Aspergillus flavus*, 80.0±0.35% for *Fusarium chlamydosporium* and 73.6±0.11% for *Aspergillus niger* as against weight reductions of 19.40±0.14%, 12.60±0.10%, and 19.40±0.18% for the same isolates respectively in the absence of cassava peels in the mineral salts medium. Overall, Cassava peels remarkably enhanced LDPE degradation by all the microbial isolates.

These results are consistent with the findings of Lee *et al*. (2005), which reported that compounding petroleum based polymers such as low-density polyethylene with natural polymers such as starch, cellulose, lignin, chitin and chitosan is a significant way to accelerate polymer biodegradation. Mohd-Asharuddin *et al*. (2017) conducted a chemical and morphological study of cassava peels and found that cassava peels contain sugars in the form of polysaccharides and holocellulose; principally cassava peels contain mainly moisture, starch and fiber in the form of lignin and cellulose (Souza *et al*., 2013; Otache *et al*., 2021).

The use of organic wastes such as cassava peels, pineapple peels and coconut husk ash in the bioremediation of soil pollutants such as hydrocarbon products have been variously studied (Dhanasekaran *et al*., 2011; Patel, 2012; Al-Wasify *et al*., 2014; Kormin *et al*., 2017). Agarry and Aremu (2012) and Patel (2012) reported that these agricultural wastes can serve as adsorbents and through the process of biosorption, they can be used effectively in removing heavy metals from contaminated environment, especially waste water or simply to increase the organic matter and nutrients in the soil where they are dumped, consequently, increase the ability of the soil microorganisms to use up the carbon in the soil for energy (Onuoha *et al*., 2020). Although the use of these agricultural wastes was conducted in the laboratory, the results pointed in similar direction.

The significant weight reduction of LDPE strips with the incorporation of CPP could be due to the fact that they constitute a good source of carbon for the microbial isolates. This could have made them grow at a faster rate, leading to higher biofilm formation on the surface of the LDPE strip. Agricultural wastes have high potency for use in producing and stimulating the growth of micro-organisms (Dhanasekaran *et al*., 2011). The greater the biofilm produced, the larger the surface area of the LDPE strip colonized by these microbes. This leaves a larger area on the strips to be covered by the extra-cellular enzymes secreted by these microbes leading to creation of more pits and holes on the LDPE strip (Li *et al*., 2020; Ru *et al*., 2020). This will naturally lead to chipping or eroding away of the material, loss of polymer integrity and subsequent weight loss of the polymer (Gajendiran *et al*., 2016).

The use of cheap, abundant and ecofriendly source of biopolymer such as starch, or starch rich agricultural wastes as employed in this study can be an interesting solution to the bioremediation of synthetic polymers such as low-density polyethylene and should be explored on a larger and commercial scale.

V. CONCLUSION

This study indicates that naturally growing soil microorganisms (bacteria and fungi) from dump sites in parts of North Central Nigeria show great capacity to utilize Low-Density Polyethylene (LDPE) at different degrees.

Bacteria belonging to the genera – *Pseudomonas*, *Bacillus*, *Providencia*, *Alcaligenes*, *Enterobacter*, *Klebsiella*, *Proteus* and Fungi belonging to the genera, *Aspergillus*, *Trichoderma*, *Mucor*, *Rhizopus*, *Basidobolus* and *Microsporium* previously isolated from dumpsites in parts of North Central Nigeria were identified as efficient degraders of low-Density Polyethylene (LDPE). The fungi were found to be more efficient degraders of LDPE than the bacterial counterparts. While the most efficient fungi (*Aspergillus flavus*) degraded 56.2% of LDPE after 8 weeks' incubation., the bacterial counterpart (*Bacillus megaterium*) degraded only 29.4% of LDPE over the same period. Fungi of the genera, *Aspergillus and Fusarium* and bacteria of the genera, *Pseudomonas* and *Bacillus* had the highest capacity of degradation compared to other genera.

The addition of agricultural waste (Cassava peels) in the incubation medium significantly enhanced biodegradation activity of the microbial isolates from 29.4% - 56.2% to 71.0%-80.2% after 8 weeks' incubation. The use of agro-industrial wastes such as cassava peels a presented in this study provides a potential solution in the bioremediation of LDPE plastics in the environment; this knowledge can be applied on a commercial scale.

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